A randomized, placebo-controlled trial of doxycycline after endoluminal aneurysm repair

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Background: **The late durability of endovascular aneurysm repair (EVAR) has been limited by progressive aortic degeneration believed to be mediated by matrix metalloproteases (MMP). The goal of this study was to evaluate the effect of a MMP inhibitor, doxycycline, on EVAR.**

Methods: **Patients undergoing EVAR were randomized to doxycycline (100 mg twice daily) or placebo for 6 months following the procedure. Clinical data, blood samples, and computed tomography (CT) scans were obtained preoperatively, postoperatively (blood only), and at 1- and 6-month follow-up. Forty-four subjects were analyzed based on intention-to-treat.**

Results: **Plasma MMP-9 decreased significantly below baseline in the doxycycline (N = 20) treated patients at 6 months (−16.4% ± 20.7%, $P < .05$) while there was a nonsignificant increase in the placebo (N = 24) group (128.1% ± 73.5%). This was primarily related to changes between 1 and 6 months. In patients with endoleaks at 6 months, plasma MMP-9 increased in 83% of the placebo treated patients, but in only 14% of the doxycycline treated group ($P < .03$). Among endoleak-free patients with AneuRx or Excluder endografts, doxycycline treatment resulted in greater decreases in maximum aortic diameter than placebo treatment (−13.3% ± 3.3% vs −3.8% ± 3.0%, $P < .05$). Furthermore, doxycycline treatment significantly reduced the aortic neck dilatation at 6 months in Excluder treated patients.**

Conclusion: **There is evidence of persistent MMP release representing ongoing aortic degradation after endografting which can be inhibited by doxycycline therapy. In analyses based on the endograft used, treatment with doxycycline also demonstrated evidence of increased aortic dimensional stability, a surrogate marker for long-term success of EVAR. Although encouraging, these results require confirmation in larger patient populations. Doxycycline should undergo more thorough evaluation as a potential adjuvant treatment to improve the results of EVAR, particularly in certain subgroups. (J Vasc Surg 2008;48:519-26.)**

Abdominal aortic aneurysms (AAA) develop as a result of arterial wall matrix degeneration, and pose a significant risk for fatal rupture when the diameter exceeds 5.5 cm. The development of endoluminal exclusion of abdominal aortic aneurysms (EVAR) has become widely adopted as a means of reducing the risk of aneurysm rupture, but late loss of effective aneurysm exclusion and need for costly follow-up and reintervention has tempered the enthusiasm for the procedure.

Ongoing aortic wall degeneration and dilatation may result in failure of AAA exclusion. Studies in human tissue and animal models strongly suggest that enzymes of the matrix metalloprotease (MMP) family play a central role in the matrix degeneration of the aorta. There is evidence both in the animal model and in clinical trials that doxycycline, a known metalloprotease inhibitor, can inhibit AAA formation and progression.[1-5]

We hypothesized that the durability of endovascular aneurysm repair might be improved by inhibiting the process of aortic degeneration with adjuvant doxycycline therapy. We designed this study to determine the short-term effects of doxycycline on both radiographic and serologic markers of aneurysm degeneration and endograft stability.

METHODS

Patient enrollment. Patients were consented and enrolled at the time of their clinic visit or admission to the hospital for a planned EVAR under Institutional Review Board (IRB) guidelines. The inclusion and exclusion criteria are listed in Fig 1. Demographic, risk factor, and medication regimen data were obtained from the patient's clinical chart at the time of enrollment. Anticipated enrollment was 76 patients.

Randomization and follow-up. Patients were randomized to over encapsulated doxycycline therapy (100 mg taken twice daily) or a placebo which was identical in physical appearance (size 00 capsules). Enrollment of women and minorities was proportional to the frequency of the disease in these populations. Randomization was performed in the pharmacy utilizing a pre-assigned table of codes. The patients received their first dose of study medication on the day following surgery and continued the study therapy for 6 months. Clinical data and peripheral

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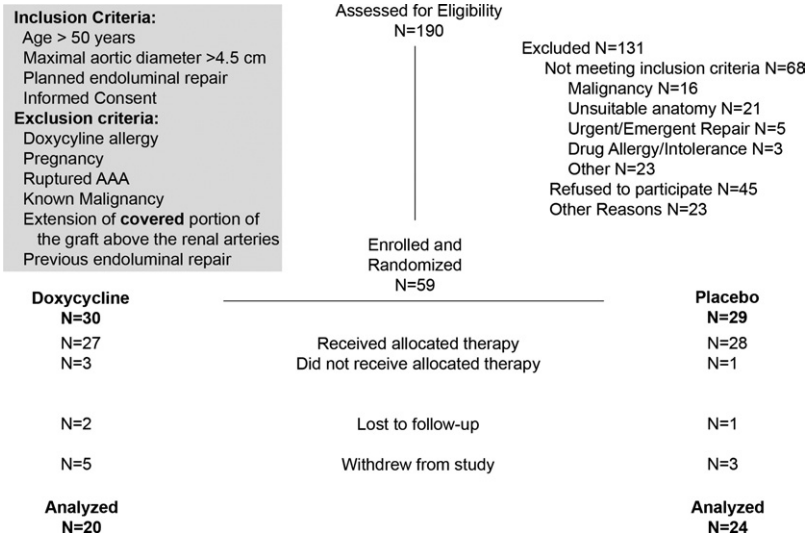

Fig 1. Study enrollment data. Inclusion and exclusion criteria for enrollment are as listed at the conclusion of the study. During the first year of enrollment, the maximal aortic diameter necessary for enrollment was 5.0 cm; this resulted in most of the anatomic exclusions. After randomization, but before dispensing the study medications we had four withdrawals: one developed a postoperative ileus and was unable to take the medication. One patient had an open abdominal aortic aneurysms (AAA) repair, and one patient from each group decided not to accept the study medications after surgery and withdrew. After the medications were dispensed, we had an additional 11 patients without 6 month follow-up. Two (1 doxycycline and 1 placebo) patients withdrew claiming medication gastrointestinal (GI) side effects, 4 developed other illnesses and declined further participation, and 2 withdrew without stating a reason, 1 person withdrew due to a change in insurance coverage and did not return to our facility, 1 patient in the placebo group died from complications of chronic obstructive pulmonary disorder, and there was 1 patient whom we could not contact and was lost to subsequent follow-up.

blood samples were collected preoperatively, immediately postoperatively, and at scheduled 1- and 6-month follow-up visits. Cross-sectional imaging was obtained for all patients preoperatively and at the 1- and 6-month follow-up visits.

GI side effects occurred with similar frequency in both the doxycycline (6) and placebo (4) treated patients, and only one patient from each arm withdrew as a result of these (P = NS). Photosensitivity developed in significantly more doxycycline treated patients (6 vs 0, $P < .01$), however, was not severe enough to cause study withdrawal in any of the patients. One patient in the placebo group died of an unrelated illness (pulmonary) during the study period.

Assays for circulating biomarkers. Serum and plasma were separated into aliquots and stored at −80°C until assayed. Measurements of the circulating biomarkers were performed with commercially available assays for plasma MMP-9, plasma MMP-2, serum IL-6, and serum IL-8 (R&D Systems, Minneapolis, Minn). Analysis was performed in a multiplex bead analysis format (Liquichip, Qiagen, Valencia, Calif). We also measured C-reactive protein (CRP) using a highly-sensitive enzyme-linked immunosorbent assay (ELISA) (hs-CRP, Bio-Check, Burlingame, Calif). The primary biomarker endpoint was the change in plasma MMP-9 concentration at 6 months compared with the baseline. Secondary endpoints evaluated were the change in circulating MMP-2, IL-6, IL-8 and CRP at 6 months compared with the baseline. Based on the pattern of circulating proteases seen during analysis of the data, we also elected to perform analysis of the effects of doxycycline treatment on the interval assessments.

Analysis of imaging data. Data were collected from the computed tomography (CT) scans, by individuals blinded as to treatment group, regarding maximal aneurysm diameter and transverse neck diameter at the level of the most inferior renal artery and at 5 mm, 10 mm, and 15 mm below that level. The mean of these four diameters was considered the neck diameter. "Centerline-of-flow" measurements were used to assess these diameters when digital data was available. All documented endoleaks and their type were recorded. The primary imaging endpoint was the change in maximal aortic diameter at 6 months. Secondary analysis was performed on the percent graft oversizing at the aortic neck and the presence or absence of endoleak at 6 months.

Statistical analysis. Data are reported as the mean ± standard error of the mean (SE). Power calculations were performed a priori on the primary endpoints of MMP-9 for circulating markers and maximum aneurysm diameter which indicated analysis of about 40 individuals per group would be necessary to have a β-error of less than .1 with a α-error of less than .05. Data representing aortic diameters and measured plasma and serum values were analyzed for the normality of the data distribution with the Shapiro-

Table I. Demographics of doxycycline treated and control study populations

	Doxycycline n = 20	*Placebo n = 24*	P *value*
Age (y)	68.9 ± 1.68	74.0 ± 1.5	.0249
Gender (% males)	80%	79.2%	
Body mass index (kg/m^2)	29.6 ± 1.5	27.5 ± 0.8	
Hypertension	18 (90%)	19 (79.2%)	
Diabetes mellitus	2 (10%)	3 (12.5%)	
Coronary disease	12 (60%)	11 (45.8%)	
Inflammatory AAA	0	1 (4.2%)	
Family history AAA	1 (5%)	3 (12.5%)	
Peripheral vascular disease	8 (40%)	7 (29.2%)	
Chronic obstructive pulmonary disease	6 (30%)	10 (41.7%)	
Current smoker	13 (65%)	7 (29.2%)	.0324
Renal insufficiency	2 (10%)	6 (25%)	
Intestinal bleed	4 (20%)	3 (12.5%)	
Aortic diameter (mm)	57.2 ± 2.1	57.2 ± 2.4	
Hemoglobin (g/dL)	13.9 ± 0.5	13.9 ± 0.3	
Creatinine (mg/dL)	1.4 ± 0.3	1.3 ± 0.2	

Wilk W Test. Statistical analyses for non-normally distributed data sets (MMP-9, MMP-2, IL-6, IL-8, and CRP) were performed on log transformations of those data sets.[6-8] Associations between plasma measurements and aneurysm size were analyzed with Pearson correlation coefficients. Comparisons of means were performed with one-way analysis of variance (ANOVA). Repeated measures multivariate analysis of variance (MANOVA) for change in measurements over time were performed where appropriate. The χ^2 test was used to compare categorical data (Fisher exact test for average cell counts less than 5). A $P < .05$ was considered statistically significant.

We also identified unanticipated important effects on the imaging endpoints based on the endograft device used as well as the presence or absence of an endoleak. Therefore, we performed post-hoc sub-group analyses of both the primary and secondary endpoints by the endograft device. Due to the small size of the female and minority groups in this study, meaningful independent statistical analysis of these groups was not informative.

RESULTS

Patient population and baseline studies. Patients were enrolled over a 2-year period and evaluated at 1 and 6 months after elective placement of an aortic endograft for an infrarenal AAA. Of the 59 patients who met the enrollment criteria and were randomized into the study, 44 subjects had either imaging or plasma marker data available at 6 months of follow-up and were included in the data analyses (Fig 1). All patients with available data were analyzed by assigned treatment group regardless of compliance with the medication regimen. The demographic characteristics of those who were randomized and completed follow-up are listed in Table I.

Although there were no significant differences between doxycycline and placebo treated subjects in the distribution of most of the demographic features, the patients in the doxycycline treatment group tended to be slightly younger and were more likely to be current smokers at the time of enrollment. Analysis of circulating markers and maximal aneurysm size did not demonstrate any significant association with either of these demographic features.

Table II. Study parameters at baseline

	Doxycycline N = 20	*Placebo n = 24*
Aortic diameters (mm)		
Maximum	57.2 ± 2.1	57.2 ± 2.4
At lowest renal artery	22.5 ± 0.6	23.4 ± 0.5
5 mm inferior	22.5 ± 0.9	23.6 ± 0.7
10 mm inferior	24.1 ± 0.7	23.4 ± 0.6
15 mm inferior	24.6 ± 0.7	24.0 ± 0.7
Circulating markers (pg/mL)		
MMP-9	45380 ± 15238	57860 ± 13910
MMP-2	199570 ± 36919	211492 ± 33702
IL-6	3.8 ± 3.1	6.6 ± 2.9
IL-8	1.9 ± 1.1	3.0 ± 1.0
CRP	56.0 ± 13.3	47.5 ± 12.2

There was no significant difference in maximal aortic diameter or the diameters of the aorta measured at the "neck" of the aneurysm. There were also no differences in preoperative circulating markers (Table II). As shown in Fig 2, there was a significant positive correlation between the preoperative maximum aortic diameter and the preoperative plasma MMP-9 level (adj. $R^2 = 0.25$, $P < .0003$), as well as a weak correlation with plasma MMP-2 (adj. $R^2 = 0.08$, $P < .04$). We saw no similar correlation between aneurysm size and any of the other serologic markers measured.

The device used for the endoluminal repair was left to the discretion of the operating surgeon. Eight patients (18.2%) were treated with an AneuRx endograft (Medtronic, Minneapolis, Minn), 19 (43.2%) with Excluder (Gore, Flagstaff, Ariz) and 17 (38.6%) with Zenith (Cook Medical, Bloomington, Ind). There were no significant differences in the device used and the baseline preoperative aortic measurements.

Procedural and periprocedural events. There were no significant differences in the proximal nominal diameter of the device used, procedural time, blood loss, or postoperative clinical laboratory values between the subjects randomized to doxycycline or placebo (Table III). Four patients required hypogastric artery occlusion. Two patients had a type II endoleak documented on the completion arteriogram. There were no perioperative mortalities, and one patient developed postoperative atrial fibrillation and myocardial ischemia evidenced by an increase in troponin.

Compared with the baseline, on the day following EVAR, we found that there were significant increases in serum IL-8 (5.8 ± 1.1 pg/ml vs 2.5 ± 1.1 pg/ml, $P < .003$), IL-6 (46.3 ± 4.5 pg/ml vs 5.3 ± 4.4 pg/ml, $P < .0001$), and CRP (137.9 ± 12.5 pg/ml vs 51.4 ± 12.3

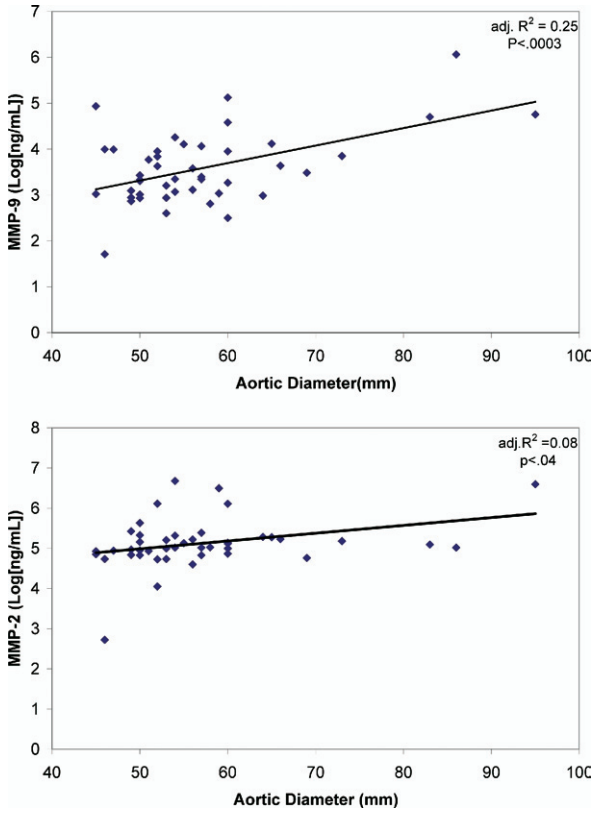

Fig 2. Correlation between pre-operative matrix metalloproteases (MMP)-9 and MMP-2 and maximum aortic diameter. Although the adjusted correlation coefficient (adj. R^2) for MMP-9 is considerably greater than that for MMP-2, circulating levels of both enzymes significantly correlate with the magnitude of aneurysmal dilatation.

Table III. Procedural parameters and periprocedural demographic data

	Doxycycline *n = 20*	*Placebo* *n = 24*
Hemoglobin (g/dL)	11.7 ± 0.4	11.1 ± 0.3
Creatinine (mg/dL)	1.3 ± 0.3	1.2 ± 0.3
Procedure time	2:44 ± 0:16	3:05 ± 0:16
Proximal device diameter (mm)	27.4 ± 0.4	26.4 ± 0.6
Blood loss (mL)	350.0 ± 64.0	406.3 ± 51.6
Fluids (mL)	2400.0 ± 209.1	2620.8 ± 224.2
Device brand	AneuRx = 3 (15%) Excluder = 10 (50%) Zenith = 7 (35%)	AneuRx = 5 (20.8%) Excluder = 9 (37.5%) Zenith = 10 (41.7%)

pg/ml, $P < .0001$). The increase in MMP-9 (90.7 ± 12.1 ng/ml vs 52.2 ± 11.9 ng/ml, $P < .06$) did not quite reach significance. There was no significant change in plasma MMP-2 levels, and there was no correlation between aneurysm size and postoperative plasma MMP-9 or MMP-2 levels.

Effect of doxycycline therapy on endoleak and maximum aortic diameter. For all patients, at 1 month, the mean AAA size was 57.1 ± 1.5 mm and the median change in AAA diameter was 0.5 mm (mean: 0.02 ± 0.6 mm). At 6 months of follow-up, the mean maximum diameter of the AAA in the study was 52.6 ± 1.7 mm. Overall, the median absolute decrease in the aortic diameter at 6 months compared with preoperatively was −2.6 mm (mean: −4.7 ± 1.0 mm, $P < .0001$), and compared with 1 month was −4.0 mm (mean: −4.8 ± 0.8 mm, $P < .0001$). The absolute changes in aortic diameter following endograft placement were dependant on the initial aortic diameters (adj. $R^2 = 0.34$, $P < .0005$).[9] At 1- and 6-month follow-up, there were no identified type I, III, or IV endoleaks, and there were 11 patients at 1 month and 12 patients at 6 months who had type II endoleaks identified.

When analyzed by treatment group, there was no significant effect of doxycycline on the primary outcome measure of change in maximal aneurysm size between baseline and 6-month measurements. There was also no effect of doxycycline therapy on the presence of an endoleak at 6 months, one of our secondary outcome measures.

It has recently been demonstrated that aneurysm shrinkage rates may be affected by the presence of an endoleak or the specific endograft chosen which may have obscured an effect of drug therapy.[10] While our study was underpowered to identify effects of these factors, device-dependent differences in the maximal diameter change between 1 and 6 months were consistent with published comparisons,[11] tending toward a greater decrease in AAA diameter for those patients treated with a Zenith (Cook) endograft (−10.3% ± 2.5%) than those with an AneuRx (Medtronic) (−8.7% ± 3.4%) or Excluder (Gore) (−6.7% ± 2.2%, P = NS).

Based on these studies, we performed post-hoc subgroup analysis, excluding patients with the Zenith endograft or an endoleak at 6 months (Fig 3). We found that between 1 and 6 months, the doxycycline treated patients who had either an AneuRx or Excluder placed had significantly greater decreases in aortic diameter than the placebo treated patients (−13.3% ± 3.3% vs −3.8% ± 3.0%, $P < .05$).

Effect of doxycycline therapy on aortic neck diameters and graft migration. The aortic neck size was expressed as the percentage by which the nominal proximal graft diameter exceeded the measured aortic diameter on imaging. The mean oversizing of the endograft at baseline overall was 15.2% and decreased to 8.3% at 6 months ($P < .0001$). We found no difference in the planned oversizing at baseline based on endograft used: 18.3% ± 1.5%, 16.5% ± 1.5%, and 12.6% ± 1.9% for the AneuRx, Zenith, and Excluder grafts, respectively (P = NS).

There was no overall difference in change in the mean graft oversizing at the aortic neck based on doxycycline therapy. At 6 months, in the placebo group, relative residual oversizing had been reduced to 8.6% ± 3.1%, 7.6% ± 2.2%, and 5.3% ± 3.3%, for AneuRx, Zenith, and Excluder endografts, respectively. This represented a significant in-

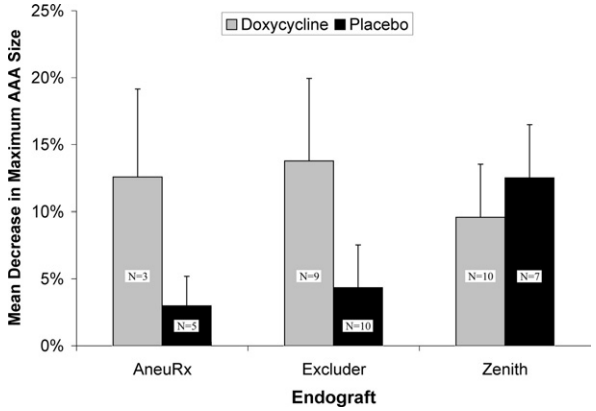

Fig 3. Effect of doxycycline treatment on maximal aneurysm diameter change after endoluminal exclusion of an abdominal aortic aneurysm. Change in maximal diameter was calculated as a percentage change in the maximum infrarenal aortic diameter between the 1 and 6 month computed tomography (CT) scans. Patients with evidence of a type II endoleak on the 6-month CT follow-up were excluded from this analysis. Doxycycline treatment resulted in a significantly greater decrease in maximal aneurysm size among the combined group of AneuRx and Excluder treated patients ($P < .05$).

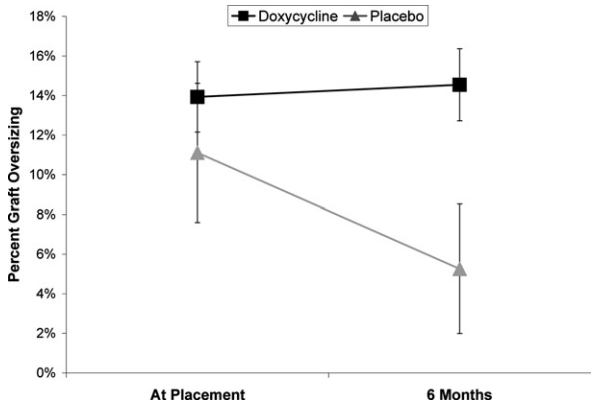

Fig 4. Effect of doxycycline treatment on aortic neck diameter among patients with Excluder endograft. In the placebo treated group all aortic necks significantly increased in diameter between the preoperative and 6-month computed tomography (CT) scan measures. Among the patients treated with an Excluder endograft, doxycycline prevented this increase in aortic neck diameter. The treated patients demonstrated a significantly smaller increase in aortic diameter than the untreated patients for this sub-group ($P < .05$). Data is expressed as a percentage of the aortic neck diameter as measured on cross-sectional imaging relative to the nominal endograft diameter (percentage oversized).

crease in neck diameter over that interval ($P < .0002$) without any significant difference between devices. Among the patients treated with doxycycline, there was an overall significant increase in neck diameter over the course of the study ($P < .02$), however, there was also a significant difference based on the device used ($P < .01$). Specifically, we found that there was no difference in the mean residual

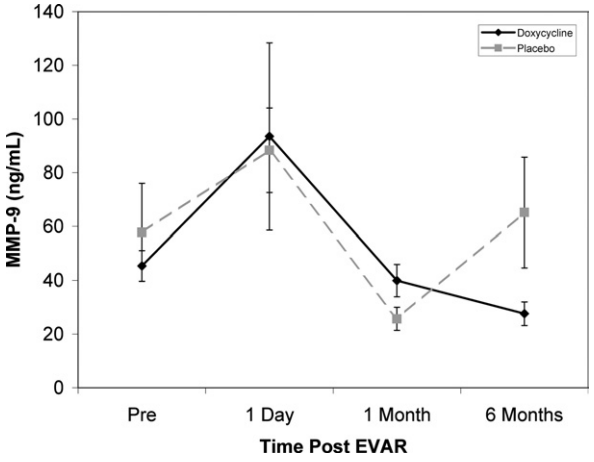

Fig 5. Effect of doxycycline therapy on the circulating levels of matrix metalloproteases (MMP)-9. The mean levels of plasma MMP-9 are plotted before and at all follow-up intervals after placement of an aortic endograft. While the surgical procedure caused an initial increase in plasma MMP-9, these levels decreased over the first postoperative month in both groups. While plasma MMP-9 subsequently increased by 6 months in the placebo treated patients, doxycycline treatment resulted in a continued decrease of circulating MMP-9 in this interval. At 6 months, plasma MMP-9 decreased significantly below preoperative levels in the doxycycline treated group ($P < .03$), but not in the placebo treated patients. The overall change in MMP-9 during the study was significantly different between treatment groups ($P < .009$).

oversizing at 6 months between subjects treated with either the Zenith (4.5% ± 2.9%) or AneuRx (6.3% ± 4.7%) grafts but significantly less dilatation of the neck in the Excluder treated patients (14.5% ± 1.8%, $P < .04$). Among the individuals with an Excluder graft, there was a significant increase in neck diameter by 6 months in the placebo treated patients ($P < .02$), while there was no significant difference between baseline and 6 months with doxycycline treatment (Fig 4).

No significant difference in the migration between the different endograft devices or based on treatment group was identified.

Effect of doxycycline therapy on plasma proteases. The primary endpoint with respect to circulating biomarkers was achieved in the doxycycline treated group where MMP-9 levels were found to decrease significantly from preoperative levels (−16.4% ± 20.7%, $P < .03$) at 6 months (Fig 5). There was no significant difference between preoperative and 6-month MMP-9 levels among the placebo treated patients (128.1% ± 73.5%).

Following the initial increase in levels the day following endograft placement, there was an overall reduction in mean plasma MMP-9 levels for all patients between postoperative day 1 and 1 month ($P < .0006$). While between 1 and 6 months, the mean MMP-9 levels in the placebo treated subjects significantly increased (206% ± 98.5%, $P < .04$), in the doxycycline treated subjects, the plasma MMP-9 levels were stable or decreased (−11.8% ± 12.5%,

$P < .06$), which is a significant difference ($P < .009$) between treatment groups. Although there was a generally positive correlation between the change in MMP-9 and the change in aortic diameter, this was not statistically significant.

In the placebo treated group, the presence of a type II endoleak was associated with a greater likelihood of increased plasma MMP-9 levels at 6 months compared with the baseline in patients without an endoleak (83% vs 35%, $P < .04$). Treatment with doxycycline reduced the frequency of increase in MMP-9 associated with an endoleak to 14% ($P < .03$ vs placebo), which was similar to the incidence without an endoleak (17%). Plasma MMP-2 levels, on the other hand, showed no significant difference based on treatment group or endoleak status and were stable throughout the study period.

Effect of doxycycline on circulating inflammatory markers. Following the acute postoperative increase, the levels of IL-6, IL-8 and CRP all significantly decreased by 1 month. The serum levels of IL-6 and CRP were not significantly different than preoperative levels at both 1 month (7.8 ± 3.4 pg/ml and 76.8 ± 11.5 pg/ml, respectively) and 6 months (4.5 ± 1.8 pg/ml, 51.1 ± 8.0 pg/ml, respectively), although the mean concentrations continued to drop in that interval. This pattern was identical for the doxycycline and placebo treated groups. The IL-8 levels remained significantly elevated at 1 month compared with preoperative levels (6.6 ± 1.4 pg/ml, $P < .02$). By 6 months, the mean IL-8 levels were no longer significantly different (4.5 ± 1.2 pg/ml). The responses of the treated and untreated patients were also similar with respect to IL-8.

DISCUSSION

Endoluminal aneurysm repair relies on the radial force of the stent against nondilated segments of aorta to maintain the proper apposition of the graft at its attachment sites. The loss of close apposition can result in the development of a leak into the aneurysm sac, repressurization of the sac, and risk of rupture. Continued degeneration of the proximal attachment site has been demonstrated after EVAR and threatens the durability of the repair.[12] Overall, long-term follow-up has demonstrated the need for occasional secondary interventions after EVAR, although the frequency is likely shrinking due to modifications and improvements in endoluminal device design. Nevertheless, it continues to be necessary to follow these patients with serial imaging.[13,14]

Matrix degrading enzymes of the MMP group, and especially MMP-9, are known to be present and active in aneurysm wall.[15] Two studies have demonstrated that plasma MMP-9 is increased in the presence of an endoleak.[8,16] It has been proposed that doxycycline therapy may slow or halt the progression of small aneurysms because of its ability to inhibit enzymes of the MMP family.[17] It currently is approved for use in gingivitis to reduce the activity of these enzymes on periodontal tissues.[18,19] Doxycycline can inhibit aneurysm formation in animal models,[1-3] inhibit MMP activity and production in human aneurysm tissue,[4] and is well tolerated in patients with AAA.[5] An effective medical therapy, such as doxycycline, has promise to reduce or eliminate secondary interventions and thereby the need for imaging follow-up after endograft placement, potentially improving the cost-effectiveness of this procedure. This pilot study was designed to determine whether doxycycline is an effective means to reduce circulating MMP-9 levels and accelerate aneurysm shrinkage following endografting.

In this study, the baseline levels of circulating MMP-9 were significantly correlated with aneurysm size, consistent with prior observations.[20] Like IL-6, IL-8, and CRP, MMP-9 increased immediately following placement of the endograft from an apparent generalized inflammatory response which had essentially resolved by 1 month.[21] Between 1 and 6 months following endograft placement the natural history of the mean plasma MMP-9 in the placebo treated patients was to increase. Remarkably, adjuvant therapy with doxycycline prevented the increase during this interval, and significantly reduced plasma MMP-9 below preoperative levels at 6 months, one of the study's primary endpoints.

As expected, in our placebo treated patients, the presence of an endoleak resulted in increasing levels of circulating MMP-9 significantly more frequently than in endoleak free patients. Treatment with doxycycline appeared to inhibit that effect, potentially signaling an inhibition of the matrix degradation response to the pressurization of the sac. Other markers of aneurysm degeneration including CRP, IL-8, and IL-6 did not appear to be significantly affected by doxycycline therapy.

The salutatory effects of doxycycline treatment on plasma MMP-9 levels may imply beneficial effect on aneurysm growth. However, none of the primary imaging endpoints was significantly affected by doxycycline treatment, overall. This may have been due to a lack of power related to not meeting our projected recruitment goals. There was also unanticipated variation based on the specific endograft placed.[11] This study was not adequately powered to identify differences based on graft type.

We performed a post-hoc reanalysis of the data by endograft, in particular examining the effects on the patients not treated with a Zenith endograft. In those endoleak-free patients with either AneuRx or Excluder grafts, the sac size decrease with doxycycline therapy was significantly greater than placebo. This is encouraging, albeit weak, evidence suggesting that the effects of doxycycline on MMP-9 may be directly affecting the aneurysmal dilatation.

Potentially of even more clinical significance, we also found that doxycycline therapy can significantly reduce the progressive dilatation of the aortic neck. Others have demonstrated that progressive neck dilatation occurs after endografting[22] (and open repair[23]), is device dependent[22] and is associated with risk of migration[22] and endoleak.[24] In this study, among the placebo-treated patients, there was progressive dilatation of the proximal neck of the an-

eurysm such that there was a significant decrease in the residual oversizing based on the nominal diameter of the endograft. The inhibition of this progressive dilatation in the Excluder patients treated with doxycycline may have been a consequence of lower radial force in that graft, allowing for small neck stabilization effects to be clinically evident.

These findings suggest that protease activity within the AAA wall continues despite endograft exclusion, and that doxycycline therapy may inhibit this degenerative process. The greatest response to doxycycline treatment was seen with the placement of the Excluder where there was evidence of accelerated aneurysm shrinkage and decreased proximal neck dilatation. This may be partially related to the covering material used with this endograft, which may have transmitted more force to the aortic wall after graft implantation.[25] Prior studies have demonstrated decreased aneurysm sac shrinkage and higher endoleak rates for this endograft, and the covering of this endograft was subsequently modified to address this.[26] However, there is evidence that aneurysms treated with other endografts may undergo similar changes albeit at a more prolonged interval.[13] Therefore, the beneficial effects of doxycycline therapy seen prominently with the Excluder device in this short study may also have the potential to benefit the durability of all endografts in the long term.

This trial is the first to use doxycycline in conjunction with aortic endografting. Although the results are exciting, there are many limitations of this study design including, small size, single-institution enrollment, and short follow-up. The limited enrollment resulted in a diminished ability to assess our primary endpoints with adequate statistical power. Although we identified potentially important effects of doxycycline on the plasma protease MMP-9, the physical aneurysm effects demonstrated were based on post-hoc sub-groups. The short follow-up did not allow us to evaluate the clinically meaningful endpoint of secondary interventions.

Overall, these results demonstrate that circulating MMP-9 can be inhibited following endografting and that this may result in improved endograft performance. This study should serve as the basis for further well-controlled and designed studies to improve the treatment of AAA.

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AUTHOR CONTRIBUTIONS

Conception and design: JC, RT
Analysis and interpretation: AH, RT, JC
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Writing the article: AH, JC
Critical revision of the article: AH, PG, BR, LS, RT, JC
Final approval of the article: AH, PG, BR, LS, RT, JC
Statistical analysis: AH, JC
Obtained funding: JC
Overall responsibility: JC

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INVITED COMMENTARY

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Despite progressive improvement in endovascular device design and deployment techniques, a small but significant fraction of post-endovascular aneurysm repair (EVAR) patients continue to experience late aneurysm-related complications. In light of the recent US Food and Drug Administration Public Health Notice regarding increasing late aneurysm-related mortality following EVAR (http://www.fda.gov/cdrh/safety/031808-medtronic.html), this prospective, randomized trial of adjuvant doxycycline therapy to improve procedural durability seems particularly timely. While providing tantalizing insights into potential mechanisms of interest, this study does not clarify the potential value of doxycycline as either adjuvant or primary therapy for abdominal aortic aneurysm (AAA) disease.

Primary study endpoints included (1) a reduction in plasma matrix metalloproteinases (MMP)-9 levels with doxycycline therapy and (2) a reduction in aneurysm sac size at 6 months. Secondary endpoints included plasma MMP-2, serum IL-6, IL-8, and highly-sensitive C-reactive protein (hs-CRP) levels at 6 months. Of these, only plasma MMP-9 levels were noted to decrease in doxycycline-treated patients between 1 and 6 months following EVAR. Doxycycline-induced reductions in plasma MMP-9 levels did not translate into reduced AAA sac size. While endograft-specific outcome analyses suggested evidence of enhanced aneurysm sac and neck stability with doxycycline, these were post-hoc analyses to be interpreted with caution. These types of analyses are best used to generate additional hypotheses for further study.

Prior studies (including reference 16 of the article above) have suggested that post-procedural plasma MMP levels are reduced following either open surgical repair or EVAR, particularly in the absence of endoleaks. In placebo treated patients in the current series, interestingly, plasma MMP-9 levels actually rose slightly following endografting. Why these plasma responses to EVAR should differ between these series is unclear. This also highlights the uncertain clinical relevance of reduced plasma MMP-9 levels in the absence of significant structural effects: in reference 5, plasma MMP-9 levels were reduced following oral doxycycline therapy in small AAA patients even though no influence was noted on ultrasound-determined diameter enlargement. Clearly more conclusive data is needed, requiring much larger treatment groups appropriately stratified by size, endoleak status, device type and exclusion methodology (eg, uni-iliac vs bifurcated device, etc), thrombus size and location and all other variables known or suspected to influence late aneurysm remodeling and regression following EVAR.

Most importantly this intriguing study underscores the need for rigorous and fully-powered trials to test the ability of doxycycline to suppress mural proteolysis in AAA, either following EVAR or as primary therapy to limit progression of early disease. More than 10 years after the original studies suggesting potential efficacy, fully-funded clinical trials are long overdue and clearly justified. Patients deserve to know whether or not doxycycline therapy will reduce their risks of death and disability from AAA disease.